Biochemical and biological mode of action of ecdysone agonists on the spruce budworm

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Abstract: The mode of action of RH-5992 (tebufenozide), a non-steroidal ecdysone agonist, on the eastern spruce budworm, *Choristoneura fumiferana*, was investigated. This diacyl hydrazine compound, upon ingestion, initiates a precocious incomplete molt that is lethal in most lepidopteran larvae including the spruce budworm. This was found to be induced when the larvae ingested the compound early in the stadium prior to the appearance of the ecdysone peak in the hemolymph. The larvae stopped feeding within 8 h post feeding (PF) and remained quiescent just as they do in preparation for a normal molt. Head capsule slippage started at 12 h PF, became pronounced by 24 h, and by 48 h an untanned new head capsule was visible behind the old one. The lack of tanning of the new cuticle was due to the failure of dopadecarboxylase gene expression. Although the old cuticle was loose around the entire body, indicating that apolysis had occurred, there was no evidence of ecdysis of the old cuticle, suggesting that eclosion hormone was probably not released. The transcription factor, *Choristoneura* hormone receptor 3 (CHR3), which is normally expressed at the onset of the hemolymph ecdysone peak, was expressed in the epidermis 1 h PF of RH-5992 confirming that this analogue acts through the ecdysone receptor system. This unique mode of action at the molecular level of this ecdysone agonist and its effectiveness as an environmentally benign control agent for the spruce budworm are described.

Keywords: RH-5992; tebufenozide; ecdysone receptor; *Choristoneura* hormone receptor 3

The dibenzoyl hydrazines are a new class of compound that cause precocious molting in lepidopteran larvae. The first compound in this series, RH-5849 and its more potent analogue, RH-5992 (tebufenozide) were found to mimic the action of 20-hydroxyecdysone (20E) by competing for the high-affinity binding sites on the ecdysone receptor complex.[1,2] We have been working on the mode of action of these compounds on the spruce budworm, *Choristoneura fumiferana* Clem, a serious pest of conifers in North America. The most effective route of entry of this compound into *C fumiferana* larvae was intrahoemocoelic injection, followed by ingestion. Topical applications were effective only when nonaqueous solvents, such as acetone and dimethyl sulfoxide, were used. The larvae were unable to discriminate between RH-5992-treated and untreated diet.

After feeding on RH-5992, the *C fumiferana* larvae initiated the molting process precociously. They stopped feeding within 6 h after ingestion of the compound and by 12 h post feeding (PF) head capsule slippage was evident. By 24 h PF head capsule slippage was more pronounced and by 48 h PF an untanned, wrinkled and poorly formed new head capsule could be seen underneath the old head capsule that had slipped. The old cuticle was loose around the entire body, indicating that apolysis had occurred, but there was no evidence of ecdysis of the old cuticle. The larvae stayed in this moribund stage for a few days before they died of starvation and desiccation. Electron microscopic studies showed that, soon after feeding on RH-5992, the larvae started synthesis of a new cuticle. In the epidermis, protein synthetic activity, as shown by hypertrophy of Golgi complexes, began as early as 3 h PF. Ecdysial droplets started to appear by 5 h PF and ecdysial space was seen by 7 h PF. The signs of apolysis were evident by 15 h PF. A new cuticle lacking endocuticular lamellae was formed within 24 h PF. By this time the old cuticle had already separated.[3]

To study the mode of action of this compound at the molecular level, we cloned cDNAs for the *C fumiferana* ecdysone receptor[4] (CfEcRA, Perera SC and Palli SR unpublished) and *C fumiferana* ultraspiracle (CfUSP).[5] The CfEcR and CfUSP proteins heterodimerize and bind to 20E and this ECR-USP-20E complex then binds to the ecdysone response element that resides in the promoter regions of 20E-responsive genes. We also cloned cDNAs for two ecdysone-induced transcription factors, *Choristoneura* hormone receptor 3 (CHR3)[6] and *Choristoneura* hormone receptor 75 (CHR75).[7] The cDNA probes were used to study the developmental expression and hormonal regulation of mRNAs for these four receptor genes. The CfEcR, CHR3 and CHR75 mRNAs increased in abundance during the ecdysteroid peaks for embryonic, larval, pupal and adult molts. CfUSP mRNA was present at a constant level throughout development in most of the tissues except in the midgut, where CfUSP mRNA levels increased during the pre-pupal ecdysone peak. 20E induced the expression of CfEcR, CHR75 and CHR3 mRNA but not CfUSP mRNA in CF-203 cells. The time course of ecdysone induction for each of these receptors was different. CfEcRB was induced within one hour of exposure to 20E, and reached the maximum levels by 3 h, after which it decreased and reached normal levels by 6 h, whereas CfEcRA was induced only after exposure to 20E for 3 h, reached the maximum levels by 6 h and decreased to normal levels by 12 h. CHR75 mRNA was induced within one hour of exposure to 20E, and reached the maximum levels by 3 h, followed by a decrease after continuous

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exposure to 20E for 6h. CHR3 mRNA started increasing at 3h and rose to the maximum levels within 6h; after continuous exposure to 20E for 12h, the mRNA levels started to decrease.

We used cDNA probes for these four receptors to study the molecular mode of action of RH-5992. CfECR, CHR75 and CHR3 mRNAs were induced in the midgut, fat body and epidermis of RH-5992-fed larvae. The induction pattern of these three receptors in the midgut, epidermis and fat body of RH-5992-fed larvae was exactly the same as that described above for 20E induction in CF-203 cells. As with 20E, RH-5992 also did not induce CfUSP mRNA. These results showed that RH-5992 acts in a similar way to 20E in causing gene expression events that occur in the presence of 20E. However, the end result of 20E increase was a successful molt, whereas increasing RH-5992 resulted in an incomplete molt. One of the possible reasons for this difference is the persistence of RH-5992 in the tissues. After initial positive regulation, 20E titres decrease, allowing the expression of genes that require the absence of 20E for their expression (eg dopadecarboxylase and larval cuticular proteins). On the other hand, RH-5992 titre does not decrease but persists in the cells and thus does not allow the expression of these genes, resulting in an incomplete molt.

RH-5992 caused the above effects on the *C fumiferana* larvae only if the larvae ingested the compound prior to the appearance of the endogenous 20E peak. If the compound was administered after the endogenous peak of 20E, the larvae molted normally into the next stage. However, the compound manifested its typical effects at the early stage of the next stadium. Quantification of CHR3 mRNA levels in the midgut, fat body and epidermis of the larvae treated with RH-5992 on each day of the last larval stage showed that the tissues were not becoming refractive to this compound after they were exposed to endogenous 20E. RH-5992 induced CHR3 mRNA throughout the 6th larval stage. These studies revealed two important properties of RH-5992: first, it can only initiate the molting process and cannot restart the molting process after endogenous 20E has initiated it and second, it is stable in the insect and can be carried over to the next larval stage where it can induce precocious molting.

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Chemical catalysis of the isomerisation of peroxidising herbicidal thiadiazolidines

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Abstract: Model reactions with various -SH, -OH and -NH nucleophiles in an aprotic solvent were used to characterise the nature of the enzymatic conversion of thiadiazolidines to triazolidines. This conversion results in greater inhibition of protoporphrinogen oxidase (protox). It is inferred that GST-isoforms probably promote formation of one of the intermediates.

Keywords: catalysed bioconversion; protox inhibitors; thiadiazolidines; triazolidines; GST-isoforms

5-Arylimino-3,4-tetramethylene-1,3,4-thiadiazolidin-2-ones (thiadiazolidines **I**. Fig 1) are peroxidising bleaching herbicides that act by interrupting chlorophyll biosynthesis *via* inhibiting the activity of the enzyme protoporphyrinogen oxidase (protox, EC 1.3.3.4).[1]

Bioconversion of **I** into isomeric 4-aryl-1,2-tetramethylene-1,2,4-triazolidin-5-thiones (triazolidines **II**, Fig 1) results in bioactivation, ie stronger inhibitors

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